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Research article

MUSCLE FIBER SPECIFIC ANTIOXIDATIVE SYSTEM ADAPTATION TO SWIM TRAINING IN RATS: INFLUENCE OF INTERMITTENT HYPOXIA

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ABSTRACT

The aim of the present study was to examine the influence of intermittent hypoxia at rest and in combination with long-term high-intensity swimming exercise on lipid peroxidation and antioxidant defense system adaptation in skeletal muscles differing in fiber type composition. High-intensity chronic exercise was performed as swimming training with load that corresponded to ~ 75 % $VO_{2\ max}$ (30 min·day^{-1}, 5 days·wk^{-1}, for 4 wk). Intermittent hypoxic training (IHT) consisted of repeated episodes of hypoxia (12%O_2, 15 min), interrupted by equal periods of recovery (5 sessions/day, for 2 wk). Sessions of IHT were used during the first two weeks and during the last two weeks of chronic exercise. Oxidative (red gastrocnemius and soleus, mix) and glycolytic (white gastrocnemius) muscles were sampled. Our results indicated that high-intensity swim training in combination with sessions of IHT induced more profound antioxidative adaptations in skeletal muscles than the exercise training only. This adaptation has muscle fiber type specificity and is reflected in significantly elevated superoxide dismutase and catalase activities in highly oxidative muscle only. Training adaptation of GSH system (reduced glutathione content, activities of glutathione reductase, glutathione peroxidase, NADPH-supplying enzyme glucose-6-phosphate dehydrogenase) occurred both in slow- and fast-twitch muscles. However, this process was more effective in oxidative muscles. IHT attenuated the increase in TBARS content induced by high-intensity swimming training. The test on exercise tolerance demonstrated a significant elevation of the swimming time to exhaustion after IHT at rest and after IHT in conjunction with high-intensity exercise in comparison with untrained and chronically exercised rats. These results confirmed that sessions of IHT might improve exercise tolerance and increase maximal work capacity.

KEY WORDS: Intermittent hypoxic training, swim training, oxidative stress, antioxidative system, adaptation, muscle fiber type.

INTRODUCTION

Hypoxic stimulus elicits specific molecular responses in skeletal muscle tissue (Hoppeler and Vogt, 2001; Vogt et al., 2001). Nowadays, in literature the advantages of training are performed under hypoxic conditions (e.g., «living low– training high" or "living high – training high") have been discussed widely (Levine and Stray-Gundersen, 1997; Truijens et al., 2003; Wilber, 2001). The results of these studies suggest that exercise under hypoxic conditions could possibly induce muscular and systematic adaptation, which either are absent or found to be a lesser degree after training under normoxic conditions (Clanton and Klawitter, 2001; Levine and Stray-Gundersen, 1997; Melissa, 1997).

At the same time, the practical application of altitude training shows contradictory results. Acute mountain sickness, and problems with acclimatization are believed to influence the effectiveness of altitude training (Boning, 1997). In recent years, the method of intermittent hypoxic training (IHT) has been used in sport practice (Bernardi, 2001). Training cycle consists of repeated short-term hypoxia, interrupted by equal periods of recovery. Hypoxic episodes are created by sojourns in hypobaric chambers or by breathing hypoxic gas mixtures in normobaric conditions. IHT increased the hypoxic ventilatory response, red blood cell count, and aerobic capacity (Bernardy, 2001; Clanton and Klawitter, 2001; Melissa, 1997). Some of these effects might be potentially beneficial in specific physiologic or pathologic conditions, therefore this method has been proposed for training of sportsmen, for acclimatization to high altitude, and for treatment of various diseases (Katayama et al., 2003; Kovalenko et al., 1993; Truijens et al., 2003).

It is known that strenuous exercise and endurance training causes oxidative stress in skeletal muscle and therefore can alter the proxidant-antioxidant balance (Alessio and Goldfarb, 1988; Atalay and Laaksonen, 2002; Davies et al., 1982; Powers et al., 1994). Despite extensive research over the years, the relationship between free radical generation, antioxidant enzymes and exercise in skeletal muscle remains controversial (Clanton and Klawitter, 1999; Ji, 1995). The discrepancies may be related to differences in exercise mode, intensity, duration of training program, and muscle fiber type. Skeletal muscles are highly heterogeneous. Each muscle fiber type has distinct metabolic characteristics and oxidative potential as well as antioxidant defense capacity (Ji, 2000), therefore it is possible to expect the fiber specific adaptive responses to intermittent hypoxia. Recent studies have demonstrated the influence of IHT on intracellular prooxidant-antioxidant homeostasis (Bailey et al., 2001; Gulyaeva et al., 1997). However evidences concerning the IHT influence on antioxidant system of skeletal muscles especially under physical exercise are uncertain.

The aim of this study was to examine the influence of IHT at rest and in combination with long-term high-intensity swimming exercise on the level of lipid peroxidation and antioxidative system adaptation in skeletal muscles differing in fiber type composition.

METHODS

The protocol of this study (application number 26/18) was approved by local Animal Research Ethic Committee. Male Wistar rats (3 mo of age at the beginning of the experiment) were used. Rats were fed with standard laboratory chow and water ad libitum and kept under artificial light-dark cycle of 12 h. The rats were randomly divided into groups as follows:

Group 1, normal control (n = 16): These rats were sedentary and under normoxic condition.

Group 2, acute exercise (n = 10): These animals were subjected to a single 30 minutes of acute swimming exercise with load that was 10±1.2% weight of body. The load was selected individually to each rat and attached to root of tail.

Group 3, chronic exercise (CE) (n = 16): In this experimental group, rats were subjected to the endurance training program consisted of swimming with load that was 10±0.8% weight of body for 30 min/day for 4 wk, 5 days·wk^{-1}. The load was selected individually to each rat every day. This level of training intensity corresponded to ~ 75 % maximal oxygen consumption ($VO_{2\ max}$) and was maintained for 4 wk.

Group 4, intermittent hypoxic training (IHT) (n = 16): These animals were subjected to intermittent hypoxia for two weeks. Hypoxic episodes were created by breathing of hypoxic gas mixtures (12%O_2) in normobaric condition in a special chamber. We used repeated short-term hypoxia (15 minutes) with normoxic intervals (15 minutes). Rats had such five sessions daily.

Group 5, CE + IHT during the first two weeks (n = 16): In this experimental group, animals were subjected to 4 weeks of high-intensity chronic exercise in conjunction with sessions of intermittent hypoxic training for the first two weeks of swimming program.

Group 6, CE + IHT during the last two weeks (n = 16): In this experimental group, animals were subjected to 4 weeks of high-intensity chronic exercise in combination with sessions of intermittent hypoxic training during the last two weeks of swimming program. Rats in groups 5 and 6 had swimming training program and sessions of intermittent hypoxia similar to groups 3 and 4.

Swimming exercises were performed in a beaker (50 cm in depth and 50 cm in wide) that was submerged in a thermostatic water bath set at 37°C. Rats swam in groups of 2-3 animals because it promotes were vigorous exercise. At the end of the training period, six rats from each of 1, 3, 5, 6 groups were selected randomly for estimation of the exercise tolerance (maximal swimming time). We used the test - swimming to exhaustion with double load. Exhaustion was determined by the inability of the rat to remain at the surface of water more than 10 s.

$VO_{2\ max}$ was measured according to Brooks and White (1978). $VO_{2\,max}$ was defined as the VO_2

Table1. Swimming time in test on exercise tolerance. Data are means (± SEM).

Groups	Control	CE (4 wk)	IHT (2 wk)	CE+IHT during the first two weeks	CE+IHT during the last two weeks
Endurance (min)	10.0 (2.0)	18.6 (1.7)*	32.4 (2.1) *,†	68.2 (1.5)*,†	63.9 (1.4) *,†

Abbreviations: CE= chronic exercise, HT= intermittent hypoxic training, CE+IHT= chronic exercise in combination with sessions of intermittent hypoxic training during the first or the last two weeks. * $p < 0.05$ compared with control group, † $p < 0.05$ between experimental groups.

after which an increase in work rate was not associated with further increase (±5%) in VO_2. At the beginning of the swimming regiment, the mean rat weights of the various groups did not differ significantly.

After the acute exercise and the test, the animals were killed immediately by decapitation. In other experimental groups, animals were killed 24 h after the last exercise training session. At the time of sacrifice, the animals were lightly anaesthetized with ether. The red muscle (red gastrocnemius and soleus, mix) and the white gastrocnemius muscle were chosen for investigations because they are actively recruited during the high-intensity swimming exercise and consist of three major locomotor muscle fiber types in the rat. Soleus includes type I fibers predominantly, whereas the red and white regions of gastrocnemius are primarily composed of type IIa and IIb fibers, respectively (Armstrong and Phelps, 1984). After decapitation, soleus and gastrocnemius muscles were removed quickly. The gastrocnemius muscle was separated further into red and white regions. Excised muscles were rapidly dissected, free of fat and tendon, divided into several portions and kept in liquid N_2. For GSH analysis, tissue samples were transferred into a medium containing 1N perchloric acid (1:10 w/v) and homogenized with motor-driven Potter-Elvehjem glass homogenizer. Resultant homogenate was centrifuged at 10,000g for 10min (4ºC). Reduced glutathione (GSH) content was measured spectrophotometrically (Sedlak and Lindsay, 1968). For the activities of enzymes and lipid peroxidation assays, the muscle samples were thawed and homogenized in 0.1 M Tris HCI buffer (pH 7.4) at 4ºC. Homogenates were centrifuged then for 15 min at 15,000g (4ºC) and the post mitochondrial supernatant was stored at -70ºC.

Lipid peroxidation (LPO) was determined by the measurement of the thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979). Superoxide dismutase (Cu, Zn-SOD) (EC 1.15.1.1) activity was determined by the method of Misra and Fridovich (1972). Catalase (CAT) (EC 1.11.1.6) activity was estimated according to Aebi (1983). Activities of tissue glutathione reductase (GR) (EC 1.6.4.2), glutathione peroxidase (GPx) (EC 1.11.1.9), glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) were determined as described previously (Carlberg and Mannervik, 1985; Deutsch, 1983; Olinescu and Nita, 1973). The protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Results are given as means ± SEM (standard error of the means) and the data were analyzed using Student's *t*- test for significant differences between experimental groups and their corresponding control groups. A level of $p < 0.05$ was accepted as statistically significant.

RESULTS

The swimming time of trained rats to exhaustion was longer than untrained rats (group1) ($p < 0.05$) and shorter ($p < 0.05$) than that had IHT alone and IHT in combination with long-term high-intensity exercise (Table 1). The test on exercise tolerance indicated that sessions of IHT improved aerobic performance and maximal work capacity in endurance trained rats.

Acute high-intensity exercise increased TBARS content in both slow- and fast-twitch skeletal muscles ($p < 0.05$). In red muscle (red gastrocnemius/soleus), we registered reduction in GSH content, in activities of SOD, GR, GPx, and elevation in CAT and G6PDH activities. Single acute swimming exercise induced a significant decrease in GSH concentration, GPx, CAT, GR activities ($p < 0.05$) and some increase in SOD activity in white gastrocnemius muscle (Figure1), (Table 2).

Chronic high-intensity exercise induced a decrease in TBARS content in muscle tissue in comparison with group 2 ($p < 0.05$). At the same time TBARS concentration remained higher than control level ($p < 0.05$). In skeletal muscle, the various antioxidant enzymes measured showed a differential response to swim training and these effects were fiber specific. No significant changes were found in CAT and GR activities in red skeletal muscle. In addition, SOD and GPx activities were higher by 14% and 19% ($p < 0.05$) in trained rats in comparison with sedentary rats. Both slow and fast muscles demonstrated a significant increase in G6PDH activity by 24% and 36% ($p < 0.01$)

Table 2. State of glutathione system and activity of glucose-6-phosphate dehydrogenase in skeletal muscle after acute and chronic exercise, and also after different regimes of intermittent hypoxic training (n = 10). Data are means (±SEM).

Groups		GSH	GR	GPx	G6PDH
Control					
	1	1.20 (.08)	6.43 (.31)	6.86 (.62)	1.98 (.09)
	2	.79 (.03)	4.66 (.20)	4.16 (.27)	2.21 (.12)
Acute exercise (30 min)					
	1	.90 (.04) **	5.00 (.18) *	5.04 (1.01)	2.19 (.10)
	2	.54 (.05) *	3.42 (.20) *	3.27 (.38)*	3.88 (.20) **
Chronic exercise (4 wk)					
	1	1.26 (.04)	6.50 (.46)	8.17 (.87)*	2.60 (.13) **
	2	.84 (.07)	5.93 (.24) **	5.45 (.15)*	3.45 (.15) **
IHT (2 wk)					
	1	1.58 (.10) *	8.36 (.78) *	10.82 (2.0) **	2.32 (.10) *
	2	1.16 (.09) **	6.85 (.46) **	6.03 (.40) *	3.11 (.12) **
Chronic exercise (4wk) + IHT during the first two weeks					
	1	1.39 (.06) *#	7.59 (.36) *#	9.62 (.05) *#	2.16 (.09)
	2	.90 (.06) *#	6.05 (.30) #	7.00 (.62) *#	2.43 (.12) #
Chronic exercise (4 wk) + IHT during the last two weeks					
	1	1.46 (.09) *#	7.88 (.41) *#	9.83 (1.06) *#	2.20 (.10)
	2	.98 (.04) *#	6.42 (.24) **	6.90 (.40) *#	2.35 (.10) #

Abbreviations: GSH= reduced glutathione (μmol·mg protein^{-1}), GR= glutathione reductase (nmol NADPH·min^{-1}·mg protein^{-1}), GPx= glutathione peroxidase (μmol GSH·mg protein^{-1}), G6PDH= glucose-6-phosphate dehydrogenase (nmol NADPH·min^{-1}· mg protein^{-1}), IHT= intermittent hypoxic training. 1) red gactrocnemius/soleus muscles, 2) white gasrtrocnemius muscle. * $p < 0.05$ compared with control, ** $p < 0.01$ compared with control; # $p < 0.05$ compared with chronic exercise.

respectively and some enhancement in GSH content. In white gastrocnemius muscle SOD activity did not alter, while GR and GPx activities were increased by 22%, 24% ($p < 0.05$) respectively, however, CAT activity was decreased by 17% ($p < 0.05$). Intermittent hypoxic training for 2 wk had significant effect on glutathione status in skeletal muscles. In slow-twitch muscle GSH content, GPx, GR, and G6PDH activities were greater respectively by 35% ($p < 0.01$), 37% ($p < 0.01$), 23% ($p < 0.05$), 15% ($p < 0.05$) than in control rats. In addition, we observed prominent increase in SOD and CAT activities by 34% ($p < 0.05$) and 38% ($p < 0.01$). After sessions of IHT in fast-twitch muscle, we found significantly higher GSH content (32%) ($p < 0.05$), activities of GR (32%), GPx (31%), G6PDH (29%), CAT (23%) than in normoxic rats. At the same time, SOD activity was slightly decreased (13%). These changes in antioxidant levels and antioxidative enzymes activities we registered in the presence of slightly increased in TBARS content in red as well as white locomotor muscles, but these changes were not statistically significant.

Chronic exercise in combination with sessions of IHT did not cause any significant increase in TBARS concentration in locomotor muscle. However, in slow muscle we registered a tendency towards diminishing in TBARS content after IHT during the last two weeks of chronic exercise in comparison with sedentary animals. In red locomotor muscles of rats under different regimens of intermittent hypoxia, the activities in SOD, CAT, and GSH-related enzymes as well as GSH content were markedly higher than in groups 1 and 3 ($p < 0.05$). G6PDH activity was remained close to the control level. Similar changes were demonstrated at white gastrocnemius muscle. Although in rats which had sessions of IHT for the fist two weeks of training program SOD activity was lower ($p < 0.05$) than in swimming trained rats. In addition, in fast muscle G6PDH activity was decreased ($p < 0.01$) in response to sessions of IHT during endurance training in comparison with trained rats only.

DISCUSSION

During intense physical activity, the flow of oxygen through muscle cells is greatly increased. High levels of oxygen uptake (up to 100-fold) can lead to excessive ROS generation and has been implicated in fatigue, muscle soreness, myofibril disruption (Clanton and Klawitter, 1999). We found that single high-intensity exercise induced a significant increase in TBARS content, decreases in GPx, GR activities and GSH content in both fast- and slow-twitch muscles. In white gastrocnemius muscle, we

observed a tendency to reduction in CAT activity and to increase in SOD activity. In red gastrocnemius muscle, this trend was inverted. These changes prove the development of oxidative stress in muscle tissue and following intracellular antioxidant disorders at acute exercise (Alessio and Goldfarb, 1988; Davies et al., 1982; Liu et al., 2000).

After the endurance swimming training, when rats had high-intensity exercise daily for 4 wk, concentration of TBARS in both slow- and fast-twitch muscles remained higher than control level. The indices of lipid peroxidation are in agreement with hypothesis of Alessio et al. (1988) stating that red fast-twitch muscle from the gastrocnemius had greater LPO rate than white fast-twitch muscle after endurance training. It is known that level of oxidative stress depends on the type and intensity of exercise (Criswell et al., 1993). We used long-term high-intensity exercise that has a potential to produce more radicals and damage. This is supported by observation of Alessio et al. (1988), who have noted a larger increase in TBARS after high-intensity compared with moderate-intensity running. Moreover, another potential mechanism involved in the oxidative stress response to swimming exercise could possibly be the redistribution of the blood flow, that is, elevated blood flow in heart, lung, and red gastrocnemius muscle, leading to increased mitochondrial respiration, which results in an increase in the production of ROS.

Data of the current study confirmed that antioxidant enzyme response to chronic exercise is highly muscle fiber specific (Hollander et al., 1999; Leeuwenburgh et al., 1997; Powers et al., 1994). Four-wk swimming training increased SOD activity only in the oxidative but not glycolytic muscle fibers. These findings are in agreement with previous studies, showing that training adaptation of SOD activity occurred in deep vastus lateralis (DVL) and red gastrocnemius (IIa) but not superficial vastus lateralis (SVL) and white gastrocnemius (IIb) muscles in rats (Hollander et al., 1999; Powers et al., 1994). Oh-Ishi et al. (1997) observed that SOD activity in rat soleus muscle (type I) was increased significantly with training, but the enzyme protein content and mRNA levels were not altered. Chronic exercise did not change CAT activity in slow-twitch muscle and decreased CAT activity in fast-twitch muscle. In fact, several reports have demonstrated decreases in catalase activity in both oxidative and mixed fiber limb muscles (Laughlin et al., 1990; Leeuwenburgh et al., 1994). However, in the most studies were reported about the absence of changes in muscle CAT activity with

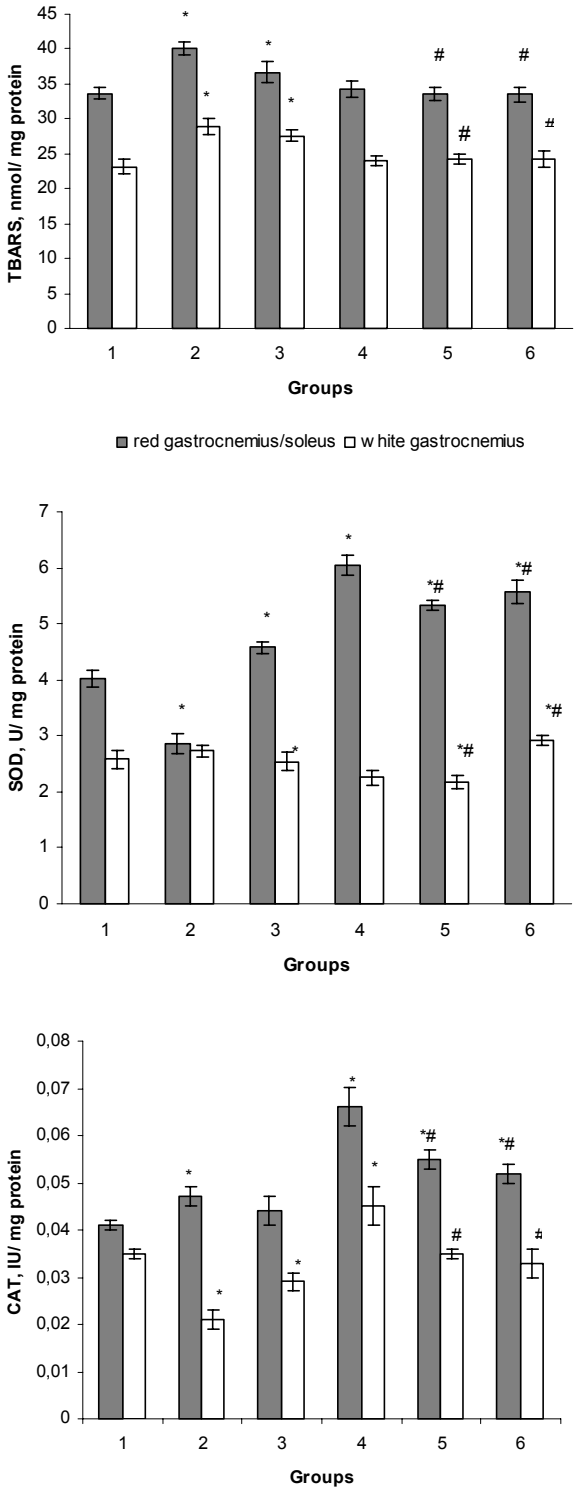

Figure1. Changes in TBARS content, activities in SOD, and catalase in rat red gastrocnemius/soleus and white gastrocnemius muscles after acute and chronic exercise, and also after different regimes of intermittent hypoxic training. Groups: 1 (control), 2 (acute exercise), 3 (chronic exercise-CE, 4wk), 4 (intermittent hypoxic training-IHT), 5 (CE-4wk + IHT during the first two weeks), 6 (CH-4wk + IHT during the last two weeks. * $p < 0.05$ compared with control group, # $p < 0.05$ compared with chronic exercise group.

training, and a few studies reported about an increase in catalase activity (Alessio and Goldfarb, 1988; Hollander et al., 1999; Ji, 1995). Our observation showed that swimming training elevated GPx activity in slow oxidative muscle, slightly increased GSH content, at the same time kept the activity of GPx at control level. All these results are in agree with previous reports (Leeuwenburgh et al., 1994; 1997). Powers et al. (1994) showed an enhancement in GPx activity in red gastrocnemius and DVL muscles, whereas soleus and white gastrocnemius muscles revealed no training effect. In contrast, endurance training did not alter the GSH content and enhanced GR, and GPx activities in white gastrocnemius in our study. These may be explained by the activation of NADPH-supplying enzyme G6PDH in both slow- (by 31%) and fast-twitch muscles (by 36%) ($p < 0.01$) to maintain intracellular reduced glutathione stores. It is known that G6PDH is the key enzyme of the pentose phosphate pathway that is responsible for the generation of NADPH and in this manner, G6PDH importantly regulates the intracellular redox balance (Salvemini et al., 1999). We used short-duration high-intensity daily swim training in which fast-twitch fibers were actively recruited (Hammond and Froelicher, 1985), therefore the response of GSH system in these muscle fibers should not be surprising.

We hypothesized that the exercise-induced oxidative stress during high-intensity swimming training might trigger adaptations in antioxidative enzymes (SOD, CAT) in oxidative muscle as well as in GSH and related enzyme systems in slow and fast muscles. This adaptation can include increased mitochondrial volume and oxidative enzymes, enhanced capillary blood volume, and myoglobin (Gul et al. 2002; 2003; Hammond and Froelicher, 1985; Phillips et al., 1996). These beneficial changes result in improved endurance and an increased maximal work capacity that was confirmed by our findings.

It is known that long-term exposure to severe hypoxia can progress cell injury, whereas repeated short-term hypoxia may initiate adaptive responses (Li and Jackson, 2002; Wenger, 2000). Acute hypoxia and especially subsequent reoxygenation induce excessive ROS generation that is typical of hypoxia-reoxygenation and ischemia-reperfusion injury in a variety of organs (Li and Jackson, 2002). Functional effects of ROS during hypoxia and reoxygenation in skeletal muscle is illustrated by the fact that antioxidant administration (particularly superoxide scavengers) result in marked improvements in contractile function during and after hypoxia (Mohanraj et al., 1998). It is considered that repeated moderate oxidative stress in hypoxia-reoxygenation episodes is an important factor in training of antiradical defense systems (Gulyaeva et al., 1997). The differences in the timing of hypoxic cycling, the length of exposure, and the degree of hypoxia of each cycle can affect on these adaptive processes. Our results showed that proposed regimen of IHT induced no significant change in TBARS content in both slow- and fast-twitch muscles in comparison with normoxic rats. This is mainly in agreement with reports of some authors about the fact that recurrent hypoxia-reoxygenation exposure attenuates ROS formation in heart muscle, hepatocytes, brain neurons (Lin et al., 2002; Vandan Hoek et al., 2000).

Changes in antioxidant defense systems induced by intermittent hypoxia have been demonstrated in animal experiments as well as in studies on human (Kovalenko et al., 1993; Sazontova et al., 1994). Some authors demonstrated tissue specific antioxidants responses to hypoxic stimulus. In rat brain, IHT resulted in an increase in SOD activity and decrease in Fe/ascorbate induced LPO (Gulyaeva et al., 1997). Sazontova et al. (1995) indicated that adaptation to interval hypoxia did not induce activation of myocardial catalase and SOD. It was shown that in red cells normobaric IHT (inhalation of gas mixture containing 10% O_2 in regime – 5 min hypoxia and 3 min normoxia for 90 min) did not affect the catalase activity and increased SOD activity by 25%. In liver *in vitro,* the intensity of LPO was decreased with the persisting activities of catalase and SOD (Sazontova et al., 1994). Unfortunately, we know only slightly, how IHT influences at prooxidant-antioxidant balance in skeletal muscle. We have found that IHT enhanced activities of SOD and CAT in slow oxidative muscle. In fast glycolytic muscle CAT activity was higher, but SOD activity was lower than control. In both muscle fiber types reduced glutathione content and activity of GSH-related enzymes were significantly greater than in normoxic and chronic exercise rats. Basing on these data we can suppose that IHT promotes an adaptation of GSH system as well as antioxidant enzymes in skeletal muscle, however the biochemical mechanisms underlying the muscle fiber specific GSH adaptation to hypoxic training is not clear. We consider that elevated level of G6PDH activity in slow- and fast-twitch muscles promotes the maintenance of intracellular GSH recycle in reducing state. Although, synthesis of GSH *de novo* and activity of γ-glutamyl cycle enzymes are important also (Leeuwenburgh et al., 1997), but without direct measurements this possibility remains speculative.

The sessions of IHT in different combinations with swimming training in red muscle reduced basal production of oxidants, greatly increased

antioxidative enzymes activities and activated of GSH system in comparison with trained rats only. In white muscle, TBARS content was lower than in chronic exercised rats but this LPO index remained slightly higher than in sedentary rats. CAT activity increased slightly in comparison with trained rats. Fast muscle adaptation in these rats was accompanied by an enhanced in GSH content and GSH-related enzymes activities. Thus, our results showed that IHT in conjunction with swim training upregulated SOD and CAT activities in highly oxidative muscle only. The training adaptation of GSH system occurred both in slow- and fast-twitch muscles. However, this process was more effective in red muscles. It was clear that sessions of intermittent hypoxia during the last two weeks of swimming training was more effective. The lack of major changes in the activation of G6PDH may be explained by the fact that intermittent hypoxia involved a shift of anaerobic glycolysis to aerobic metabolism by an increase in oxidative enzymes (Terrados et al., 1990; Yoshino et al., 1990).

It is known that hypoxic training elicits specific molecular responses in tissues including activation of a transcription factor such as hypoxia-inducible factor-1 (HIF-1), which is also expressed in skeletal muscle (Wenger, 2002). Activation of HIF-1 leads to cellular adaptation, which counteract the effects of reduced oxygen supply to cells under hypoxic conditions (Vogt et al., 2001; Wenger, 2000). These include improved oxygen transport capacity in the blood due to an erythropoietin-induced increase of the hematocrit, induction of neovascularization by an enhanced expression of the VEGF, more efficient utilization of oxygen due to an increase in glucose oxidation induced by the activation of glycolytic enzymes, intensification of protective protein synthesis including the enzymes of antioxidative defense system (Clanton and Klawitter, 2001; Wenger, 2002; Zhai et al., 1996). These and other data indicate that HIF-1 is involved in the cellular oxygen-sensing system (Wenger, 2000). Vogt et al. (2001) postulate that muscle gene expression depends on training intensity as well as on the presence or absence of hypoxia during the training session.

Hence, IHT might lead to the adaptations, which improve oxygen transport, substrate oxidation, and probably tissue growth, that are known to influence exercise performance capacity (Hoppeler and Vogt, 2001; Katayama et al., 2003; Terrados et al., 1990). In our study, the test on exercise tolerance demonstrated significant enhancement of swimming time to exhaustion after IHT and after IHT in conjunction with high-intensity exercise in comparison with control and chronic exercise rats.

CONCLUSIONS

The results of the present study confirm that IHT may improve exercise tolerance and maximal work capacity. Different combination of IHT sessions during chronic training showed that probably the main rationale for use of IHT is based on the cross-protective effect of adaptation to one type of stress, which then provides resistance to another stress type (Meerzon, 1993). However, the molecular mechanisms of these processes remain not clear.

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KEY POINTS

- Single high-intensity exercise induces a significant increase in TBARS content, decreases in GPx, GR activities and GSH content in both fast- and slow-twitch muscles.
- Intermittent hypoxic training (IHT) may improve exercise tolerance and maximal work capacity.
- Antioxidant enzyme response to chronic exercise is highly muscle fiber specific.
- IHT induces no significant change in TBARS content in both slow- and fast-twitch muscles in comparison with normoxic rats.
- IHT promotes an adaptation of GSH system as well as antioxidant enzymes in skeletal muscle, however the biochemical mechanisms underlying the muscle fiber specific GSH adaptation to hypoxic training is not clear.

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